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Production of Ginsenoside-Rg_3 from *Lipomyces starkeyi* Grown on Ginseng-Steaming Effluent

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To produce ginsenoside-Rg_3 enriched yeast from ginseng-steaming effluent (GSE), *Lipomyces starkeyi*, which tends to grow well in GSE, was cultured in sterilized GSE and its growth and production of ginsenoside-Rg_3 were determined. Growth of *L. starkeyi* was 86.1 mg per g GSE and its ginsenoside-Rg_3 contents was 0.013 mg per g GSE.

KEYWORDS : Ginseng-steaming effluent, Ginsenoside-Rg_3, *Lipomyces starkeyi*

Recently, scientists have begun to use various yeasts for the production of bioactive compounds, such as an antihypertensive angiotensin I-converting enzyme inhibitor [1, 2] and an antiangiogenic compound [3]. Ginsenoside-Rg_3 [4] and antidementia β-secretase inhibitor [5] have also been produced and characterized from *Saccharomyces cerevisiae*.

Ginseng and its extracts are used in the production of various health foods and neutriceuticals. From the manufacturing process of ginseng products there is a large amount of ginseng steaming effluent (GSE) that is discharged as waste [6]. GSE, however, contains useful ginsenosides, sugars, protein and industrial enzymes and has potential physiological functionality [7]. Only a small amount of GSE is necessary for the extraction of useful ginsenosides or in the production of bioactive malto-oligosaccharides [6], chitosan [8] and ribonucleotides [9]. The bulk of GSE is discharged into the sewage, causing environmental pollution. We studied the production of bioactive compounds from GSE and herein report on the production and characterization of ribonucleotides from *Pichia anomala* [9] and its mutant [10], chitosan by *Mucor miehei* [8] and ginsenoside-Rg_3 [4] from *S. cerevisiae* grown on GSE. We now describe production of ginsenoside-Rg_3 enriched *Lipomyces starkeyi*, using ginseng-steaming effluent.

The *L. starkeyi* used in this study was obtained from the Bioresource Center of the Korean Research Institute of Bioscience and Biotechnology (KRIBB, Daejeon, Korea). The GSE was obtained from a ginseng processing plant in Geumsan, of Chungnam province, South Korea. This GSE contained 63.8% total sugar, 34.2% crude protein, 0.2% crude fat and 1.8% ash, and it had a pH of 6.5. Ginsenoside standard products and ginsenoside analysis reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Unless otherwise specified, all chemicals were of analytical grade. *P. anomala* KCCM 11473 and *L. starkeyi*, grown on yeast extract-peptone-dextrose (YEPD) medium at 30°C for 2 days were inoculated in the GSE (pH 6.5) and cultured at 30°C for 72 hr. After centrifugation of the culture broth at 10,000 g for 15 min, the yeast cells were harvested, sonicated and centrifuged again in order to obtain the cell-free extracts.

Determination of growth and ginsenoside contents was carried out according to the method of Kim *et al.* [4], with slight modifications. Growth of *L. starkeyi* in the GSE was 86.1 mg per g GSE (0.52, A_{660}). Growth of *L. starkeyi* was lower than that of *S. cerevisiae* grown on GSE (1.38, A_{660}) [4].

We determined the ginsenoside content of cell-free extract from *L. starkeyi* grown on GSE. As shown in Fig. 1, ginsenoside-Rg_3 was identified in HPLC chromatogram of *L. starkeyi* grown on GSE and the other ginsenosides were not detected. The result was same as those of *S. cerevisiae*, *Saccharomyces pastorianus*, *Kluyveromyces fragilis* and *Zygosaccharomyces rouxii* [4]. Ginsenoside contents in *L. starkeyi* was analyzed and compared with those of GSE itself and with the yeasts grown on a YEPD medium (Table 1). Compared to the 9.66 mg of ginsenoside-Rg_3/g GSE, which was contained in GSE itself, *L. starkeyi* grown on GSE (1 g) contained 0.013 mg of ginsenoside-Rg_3/g GSE. Any ginsenosides were not dectected in cell-free extracts from the yeast grown on YEPD medium (data not shown). The ginsenoside-Rg_3 content of *L. starkeyi* was lower than that of *S. cerevisiae*, *K. flagilis* and *Zygosacch. rouxii* [4]. *L. starkeyi* is known for intracellular fat production. There is little information on the production of some bioactive compounds from *L. starkeyi;* this is the first report that ginsenoside-Rg_3 was produced from the yeast.

It is known that Rg_3 have many valuable attributes, such as anti-cancer, anti-dementia and anti-stress proper-

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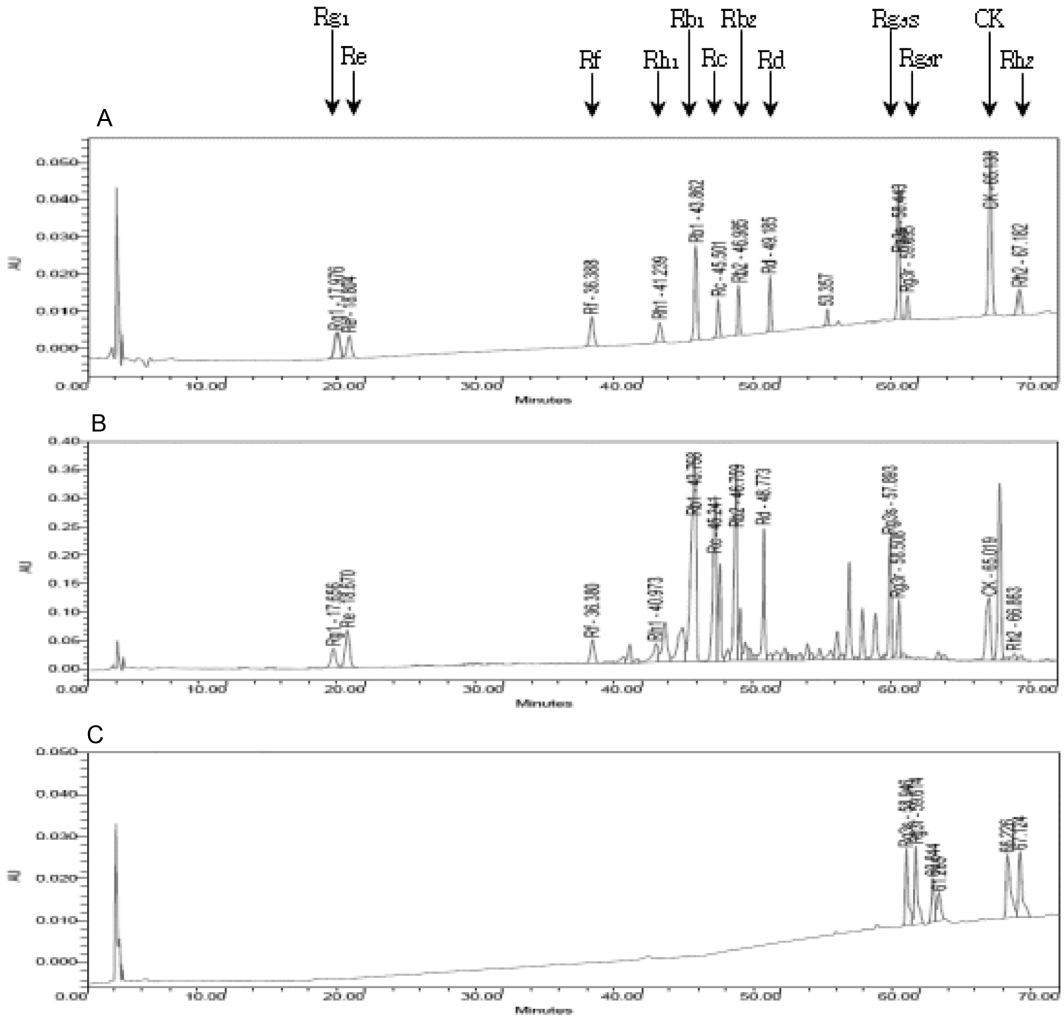

Fig. 1. HPLC chromatogram of ginsenosides. A, Ginsenoside standard; B, Ginseng - steaming effluent (GSE); C, *Lipomyces starkeyi* grown on GSE.

Table 1. Ginsenoside contents of *Lipomyces starkeyi* grown on the ginseng-steaming effluent (mg/g solid)

	Ginsenosides									
	Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1	Rg_3	Rh_2	Total
GSE[a]	29.91	15.19	16.78	7.99	4.68	2.28	2.25	9.66	1.92	90.66
L. starkeyi	–	–	–	–	–	–	–	0.013	–	0.013

GSE, ginseng-steaming effluent.
[a]Rg_3 contents of ginseng - steaming effluent itself.

ties [4]. Also, it is converted from Rb_1, Rb_2 and Rc by treatment of slight acid or heat (100°C, 20 min) [4]. It is presumed that ginsenoside-Rg_3 of *L. starkeyi* grown on GSE in this study was produced from sterilization (121°C, 20 min) of GSE or bioconversion of the yeast during cultivation. Further study is need to illustrate the Rg_3 production mechanism in these yeast.

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